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# Optical techniques for breath analysis: from single to multi-species detection

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**Abstract**. Optical spectroscopy can be used for trace level gas analysis in different applications, including exhaled breath research. A common approach is the targeted online, real-time analysis of small molecules (2-5 atoms). Currently, the methodology is normally used for the detection of single analytes at trace levels, or 2-3 species at most at the same time. The main limitation preventing sensitive multi-species detection has been the limited fast scanning range of the lasers used as light sources. This limitation is currently being eliminated by the availability of optical frequency combs (OFC) which offer wide spectral bandwidths and the benefits of a laser-type light source. Recent advances in mid-infrared OFC technology allow measurements in the so-called molecular fingerprint region of the electromagnetic spectrum where many molecules have strong fundamental vibrational transitions that enable sensitive detection. Several technical hurdles remain to overcome but if these problems can be solved, laser absorption spectroscopy has the potential to challenge mass spectrometry in online multi-species trace gas analysis.

## 1. Introduction

Optical absorption spectroscopy is one of the established methodologies currently used in exhaled breath analysis [1]. One of the main strengths of the technique lies in the selective nature of the absorption phenomenon. Transition energies for transitions between molecular energy levels are specific for every molecule due to the quantum mechanical nature of molecules. By choosing the targeted transitions carefully, measurement of optical absorption peaks enables quantitative and reliable analysis of specific compounds. Using modern optical measurement techniques, the analysis is also sensitive. It is possible to reach detection limits down to part-per-billion (ppb) and part-per-trillion (ppt) levels. Such results can be obtained without pre-concentration of the sample and the analysis can be performed both online and in real-time. The technology has also been taken outside of the laboratory. Robust, easy-to-use portable instruments are available from many commercial vendors and employed in many field applications outside of breath analysis research, from atmospheric measurements to the analysis of industrial emissions. These instruments can be operated in rough conditions and do not require an expert user.

The methodology has its drawbacks. In most current implementations, optical instruments allow the analysis of only 1-3 molecules at a time at trace level concentrations. The analysis is also limited to fairly small molecules, containing 2-5 atoms only, with some exceptions. This is in contrast to the soft chemical ionization mass spectrometry techniques, like proton transfer reaction mass spectrometry (PTR-MS) and selected ion-flow mass spectrometry (SIFT-MS). These online, real-time mass spectrometry methods allow multi-species detection at trace-level concentrations.

Most of the disadvantages of the optical absorption technique are connected with the characteristics of narrowly tunable lasers used as light sources in the instruments. Due to the emergence of optical frequency comb (OFC) technology, the limitations concerning the number and size of analytes will, to

a certain degree, most likely disappear in the future. These developments will enable optical techniques to start to challenge chemical ionization mass spectrometry techniques in state-of-the-art real-time trace level multi-species detection. However, the application of OFC spectroscopy to the analysis of exhaled breath is by no means trivial. Significant amount of work lies ahead before the technology can be used for robust and routine multi-species analysis at trace levels.

## 2. Optical techniques

### *2.1. Basic principles*

Most optical gas analysis instruments are based on vibrational absorption spectroscopy conducted in near-infrared (1-2 μm) or mid-infrared (3-10 μm) wavelength regions. In some cases absorptions in visible or ultraviolet range due to electronic transitions can also be exploited. Most molecules will exhibit strong vibrational transitions in near- and mid-infrared and only certain homonuclear molecules (for example, $N_2$, $H_2$ and $O_2$) are completely infrared inactive. This makes the absorption technique a nearly general methodology for chemical analysis. In addition to absorption, emission (fluorescence) and scattering (Raman) spectroscopy can also be employed but these methods are not commonly used in gas-phase analysis. A notable exception is the detection of NO by chemiluminescence [2]. The reaction of NO with $O_3$ produces an electronically excited state of $NO_2$ which decays via emission. The measurement of FeNO (fractional exhaled NO) for the diagnosis and monitoring of airway inflammation is one of the most successful breath tests so far. Other techniques have been developed for the breath NO detection but chemiluminescence remains the gold standard [3].

Figure 1 illustrates infrared absorption spectra of several molecules present in breath. Every one of these individual absorption lines can be, in principle, used to analyze the concentration of the corresponding species. The amount of absorbed light is proportional to the product of the absorption line strength, concentration of the species and the absorption path length. The strength of the absorption line will therefore determine, along with instrumental performance, the achievable detection limit. On the other hand, overlap with absorption lines belonging to other species present in breath will potentially cause issues with cross-selectivity. The researcher must first locate a strong absorption peak that is well separated from all other strong peaks and then find a suitable light source that matches this wavelength. Overlap with $H_2O$ and $CO_2$ lines is especially significant due to the high concentration of these species in breath. Even very weak $H_2O$ and $CO_2$ lines can induce serious overlap issues.

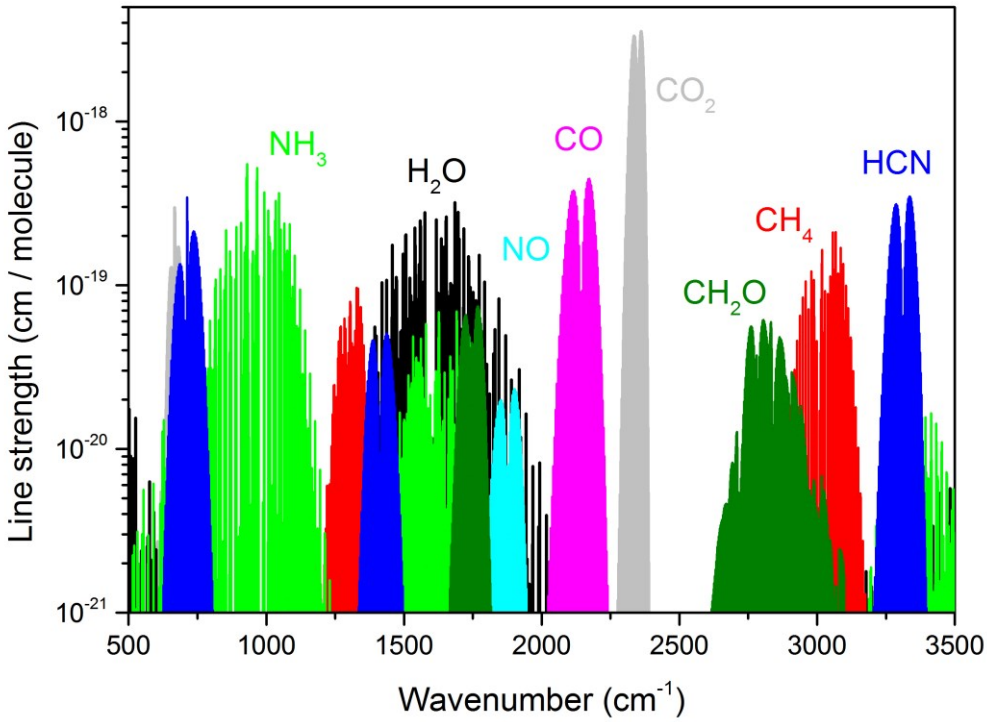


**Figure 1.** Absorption spectra of some breath biomarkers in the region between 500 and 3500 $cm^{-1}$ (20 µm and 2.9 µm). Note that the figure shows only the absorption line strengths per molecule, not taking into account the abundances of the molecular species in breath. The molecular line data are from the HITRAN 2012 database [4].

The sensitivity of the absorption measurement will depend on the specific detection technique used in the instrument. The simplest way is to send the light through the sample gas cell directly, making a single pass through the sample before the change in light intensity is recorded by a photo-detector. The sensitivity of such a direct absorption measurement will allow the analysis of only the most abundant species in breath. An example of such a single-pass absorption measurement is the use of non-dispersive infrared (NDIR) spectroscopy for the analysis of $^{13}C$ isotopologue of $CO_2$. The technology is used in the clinically successful urea breath test (UBT) for *H. Pylori* diagnosis [5]. To reach a ppb or ppt level detection limit, two methods are commonly used to increase the sensitivity. In the first one, the absorption path length is increased as the amount of absorption is proportional to the path length. This can be done by using so-called high-finesse optical cavities which consist of two or more highly reflective (> 99.99 %) mirrors. The sample cell is formed between the mirrors and the photons travel through the sample tens of thousands of times before exiting the cavity, providing an absorption path length of up to tens of kilometers [6]. Many different implementations of the general principle exist, for example, cavity ring-down spectroscopy (CRDS), cavity-enhanced absorption spectroscopy (CEAS) and off-axis cavity-enhanced absorption spectroscopy (OA-CEAS). The absorption path length can also be increased using a multi-pass cell. Compared to the cavity-enhanced techniques, this is a simpler approach and provides an absorption path length of up to tens of meters under normal circumstances. However, by combining the multi-pass cell with a laser that targets a strong mid-infrared transition, a detection limit in the low ppb-range can be achieved [7]. Another way to increase the sensitivity is to modify the fashion in which the absorption is detected. In photoacoustic spectroscopy the absorption is

detected by measuring the acoustic wave that is generated by the gas sample as it absorbs modulated light [8]. Again, different versions of the general technique exist, the difference mainly being in the type of microphone that is used for the acoustic detection. Detection can be done using a standard condenser microphone [9] but novel types of microphones have also been developed for use in photoacoustic spectroscopy. Special types of microphones are used in, for example, quartz enhanced photoacoustic spectroscopy (QEPAS) [10] and cantilever-enhanced photo-acoustic spectroscopy (CEPAS) [11].

As such, both photoacoustic and cavity-enhanced techniques provide a sensitivity that is normally good enough for exhaled breath studies. However, for multi-species detection, the absorption measurement should be performed simultaneously over a wide wavelength region to measure many absorption lines at once. In the first approximation, the wavelength range is not limited by the detection technique but rather by the light source used in the measurement. Thermal broadband light sources provide a wide spectrum but the weak light intensity limits the achievable sensitivity. For this reason, sensitive absorption instruments usually employ a laser as a light source.

### *2.2. Narrowly tunable lasers as light sources*

At present, most optical exhaled breath investigations employ wavelength tunable lasers as light sources. Using different types of lasers, absorption spectrometers can currently be made to operate almost anywhere in the near- and mid-infrared regions. Mid-infrared is especially attractive since the strongest, fundamental vibrational transitions take place in this region. Regardless of the specific wavelength, these lasers share a common trait. They are monochromatic (emit a single wavelength) and the wavelength can normally be scanned quickly only over a very limited wavelength region. As illustrated in Figure 2, this normally limits the optical detection to only one or two species at a time. The scanning time scale depends on the instrument and varies between less than one second and a couple of minutes per an optical transition line. The tuning range can be extended by using the so-called external cavity method for diode lasers (ECDL) or quantum cascade lasers (EC-QCL). Alternatively, it is possible to operate several narrowly tunable laser sources within one instrument and thus scan a broader wavelength range. Using such approaches, one can potentially probe several biomarkers at trace levels.

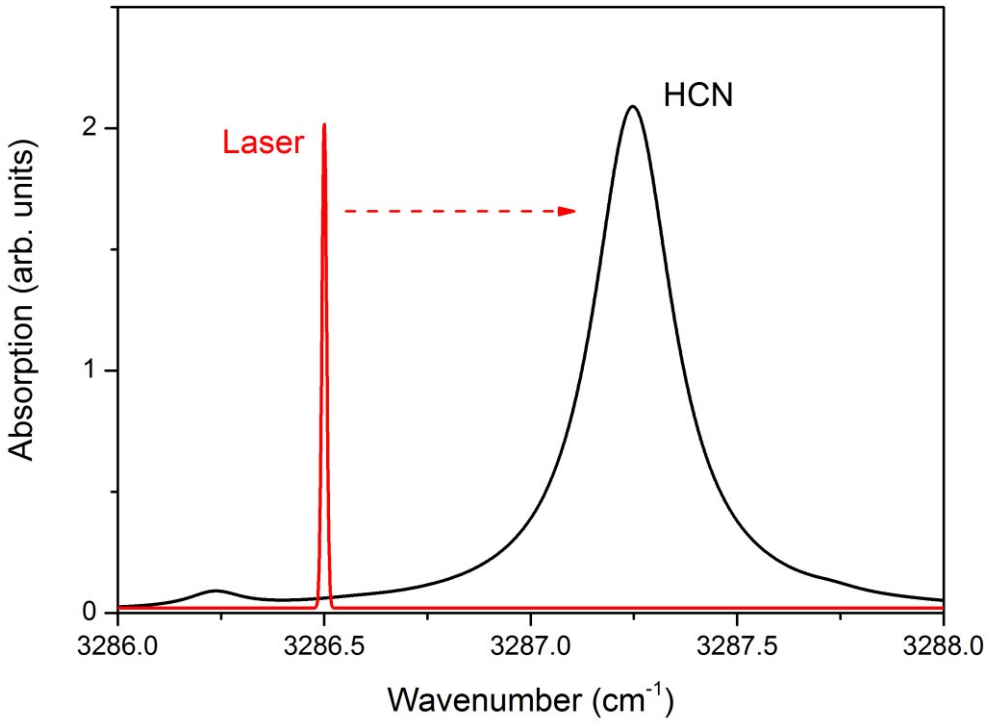


**Figure 2.** An illustration of laser scan over an individual molecular absorption line. Note that the laser line is included in the graph for illustrative purposes, the vertical axis of the graph relates only to the molecular absorption. The HCN line data are from the HITRAN 2012 database [4].

Due to the narrow scanning range, tunable lasers are often used to probe fairly small molecules. Small molecules exhibit narrow, well characterized absorption lines. Such an absorption line can be fitted to well-known line-shape functions and the concentration calculated from the peak area. Larger molecules (6 atoms or more) normally exhibit broad, complicated absorption features which cannot be contained in a typical narrow scan. In principle, one can measure the concentration by recording only a part of the absorption feature but this approach is prone to cross-selectivity issues due to overlapping peaks. Examples of laser spectroscopic studies of breath biomarkers include hydrogen cyanide [12, 13], ammonia [14, 15], methane [16], acetone [17], ethylene [18] and nitric oxide [19]. These biomarkers can be used in, for example, diagnosis of *Pseudomonas aeruginosa* infection (HCN) [13], monitoring of lipid peroxidation ($C_2H_4$) [18], monitoring of dialysis efficacy ($NH_3$) [20] and diagnosis of gastrointestinal conditions ($CH_4$) [16]. For a comprehensive list of references on laser spectroscopic breath studies, the reader is referred to the report of the task force on "Real-time spectrometry and spectroscopy" of the International Association of Breath Research [21]. Commercial instruments based on different forms of sensitive laser spectroscopy (cavity-enhanced, photoacoustic) are available from several vendors, reaching detection limits of ppb and below. The main markets for these analyzers are, for example, in environmental research, emission and process monitoring and the semiconductor industry. However, by coupling them to a suitable breath sampler, these types of instruments can in some cases be also adapted for exhaled breath research.

### *2.3. Optical frequency combs as light sources*

A typical laser emits light in a narrow wavelength region. The spectrum of an optical frequency comb (OFC) instead consists of a series of discrete, equally spaced laser lines covering a wide wavelength region [22]. The OFC technology has applications in many areas of science and engineering. One half of the Nobel Prize in Physics in 2005 was awarded to John L. Hall and Theodor W. Hänsch for their contributions to the development of OFCs.

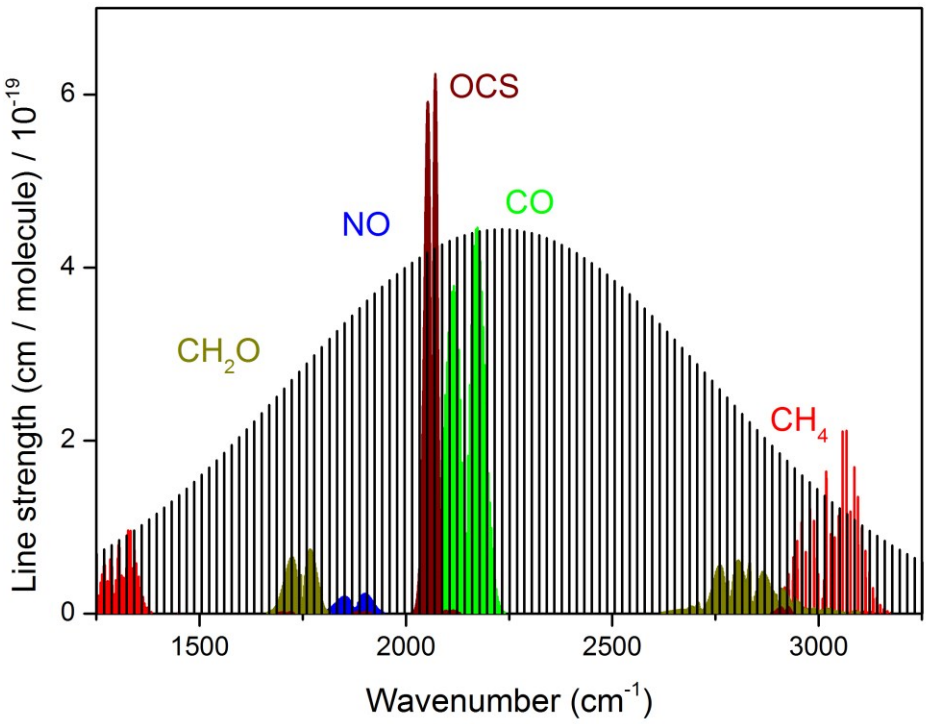


**Figure 3.** Absorption spectra of some breath biomarkers covered by an octave-spanning optical frequency comb. The frequency comb is included in the graph for illustrative purposes, the vertical axis of the graph relates only to the molecular absorption. The separation between the individual comb lines is exaggerated for visual purposes. The line strengths of the OCS lines have been divided by factor of two for clarity. The molecular line data are from the HITRAN 2012 database [4].

The wide wavelength coverage of an OFC can be used for great effect in spectroscopy. Basically, an OFC is the equivalent of a hundred thousand or one million lasers contained in one light source. Instead of scanning a narrow laser line over individual absorption peaks, an OFC can be used to cover the absorption bands of many molecules simultaneously. A so-called octave-spanning OFC covers a frequency region where the highest frequency in the spectrum is at least twice the lowest frequency. In the mid-infrared region, this is enough to cover much of the molecular fingerprint region. The principle is illustrated in Figure 3. The technology is more developed in the near-infrared region but several techniques have been devised to produce OFCs in the mid-infrared region as well [22, 23].

Although the principle of OFC spectroscopy is simple, several complications arise when trying to apply the method in practice. First one has to do with the spectral analysis of the broadband light needed to extract the molecular absorption as a function of wavelength. This can be done using traditional dispersive spectrometers. However, these can be bulky and cannot normally provide the high spectral

resolution needed for gas phase spectroscopy. Another possibility is to use a Fourier transform infrared (FTIR) spectrometer for spectral analysis. A more elegant solution that provides high spectral resolution is the dual-comb approach [22]. One OFC is first passed through the gas sample and then overlapped with another OFC. The optical frequencies interfere and the signal is down-converted to radio frequencies which are then Fourier transformed to reveal the absorption spectrum. The requirement of two OFCs is quite demanding but implementations exist where the two beams are generated from one OFC.

Another technical problem has to do with sensitivity. As with narrowly tunable lasers, the sensitivity of the instrument depends on the absorption path length. To achieve a ppt-ppb level detection limit, this means that the OFC needs to be coupled with a high-finesse optical cavity. Cavity-enhanced OFC experiments have been done but these are challenging to perform over a wide wavelength region due to the limited wavelength coverage of the high-reflectivity mirrors currently available [24].

Demonstration experiments with exhaled breath have already been performed using OFC spectroscopy [25]. However, the full potential of the technique has not yet been reached. In principle, OFC spectroscopy can deliver online, real-time multi-species detection of small and medium-sized molecules at ppt-ppb levels. In addition to the technical challenges related to OFC spectroscopy itself, the analysis of broadband infrared spectra of breath samples containing dozens of infrared active molecules is far from trivial. Multi-component analysis of infrared spectra is used in traditional FTIR spectroscopy but normally in cases where the 10-20 analytes are present at fairly high (ppm) concentrations. A breath sample will contain a large number of analytes at very low concentrations. In addition, the analysis will be complicated by the presence of few molecules ($H_2O$, $CO_2$) at very high concentrations and a few others ($CH_4$, $NH_3$, CO, acetone) at ppm and sub-ppm levels. The resulting infrared spectrum will be difficult to analyze, even more so automatically and in real-time.

## 3. Summary

Looking at the breath analysis literature, one concludes that the field is dominated by MS techniques and different types of sensors. However, the list of breath tests approved by the US Food and Drug Administration (FDA) includes quite a few optical-based measurements: urea breath test, capnography and FeNO (chemiluminescence - “semi-optical”). It is also interesting to note that, to date, most of the FDA approved tests are single-species tests. The test for heart transplant rejection (C4-C20 alkanes and monomethylalkanes) is a notable exception [26]. This is exactly where the optical methods excel in: reliable and fast quantitative analysis of single analytes.

Most researchers working in the field would, however, agree that multi-species detection is important for breath analysis, at least in the exploratory phase of the research. Here the competing technologies (MS, sensors) have traditionally held an edge over the optical methods. For online, real-time multi-species breath analysis at ppt-ppb levels, the chemical ionization -based MS techniques, PTR-MS and SIFT-MS, are probably the best commercially available technologies at the moment. Optical spectrometers based on OFCs will most likely start to challenge these MS-based instruments at some point in the future. How soon this will happen and how serious the challenge will be, will remain to be seen.

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